ULTRA-WEAK LUMINESCENCE AND OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA

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/Following is the translation of an article by Yu. A. Vladimirov and O. F. Lvova, Institute of Biological Physics, AN USSR, Moscow, published in the Russian-language periodical Biofizika (Biophysics) Vol 9, 1964, pages 506--507. It was submitted on 3 Jun 1963. Translation performed by Sp/7 Charles T. Ostertag Jr./

An experimental study of the role of electronic excited conditions in biochemical reactions may be carried out by means of investigating the ultra-weak luminescence of biological systems. This was begun by Vladimirov and Litvin in 1958 /1/.

It was shown earlier that in homogenates of animal tissues, at 37° in the presence of oxygen, a luminescence develops which is caused by unknown, probably enzymatic, oxidizing processes /2/. Later investigations, including the study of the kinetics and temperature dependency of such luminescence (the detailed results of this investigation will be published by us in the near future), showed that de-excitation of the quantum completes the sequence of processes, including extraction from the tissue, the oxidation of some unknown compound and the subsequent breaking down of the oxidized product.

In the present work luminescence was detected in the mitochondria of the liver of a rat, in which the process of oxidative phosphorylation had taken place. The mitochondria were isolated in 0.25 M saccharose at a pH of 7.4; their precipitation was carried out at 0--4°, 8,000--10,000 g. /3/. After the addition of an incubation mixture the suspension was placed in front of the window of a photomultiplier in a thermostabilized cuvette, through which oxygen had been passed which had been warmed up to the same temperature (39°).

For a long time we did not succeed in detecting the luminescence in a suspension of mitochondria, to which we had added ATP, respiration substrates and Mg^{2+}. As it was cleared up later, the absence of luminescence was caused by the fact that during the isolation of mitochondria we used saccharose with the addition of EDTA. It is apparent from the drawing that in the absence of EDTA the luminescence by far exceeds the dark background. Following the addition of EDTA the luminescence by far exceeds the dark background. Following the addition of EDTA to the incubation mixture, luminescence does not develop. The mechanism of the effect of EDTA on the development of luminescence remained unexplained, however attention is merited by the fact that EDTA suppresses oxidative phosphorylation in liver mitochondria /4/.

It was subsequently cleared up that for the development of luminescence it is important that all the components be present which are necessary for oxidative phosphorylation: Oxygen, an adenylic system, inorganic phosphate, oxidation substrates and magnesium ions. In our experiments luminescence was not observed in the absence of oxygen. As can be seen from curve b it also

did not develop at all in the absence of ATP. In the event of replacing ATP and ADP the intensity of luminescence remained the same, though it is possible its development was somewhat suppressed in time. To us this seems natural for a system in which the synthesis of ATP takes place continuously due to oxidative phosphorylation. In the absence of oxidation substrates added from without (succinic and glutamic acids) the intensity of luminescence is cut in half.

When the Mg^{2+} ions are replaced by Ca^{2+} ions in the incubation mixture, that is, under conditions when free oxidation has a significantly greater probability than when combined with phosphorylation, the intensity of luminescence dropped from 440 to 240 imp/min.

Thus, all of the above cited data testify to the fact that the most intense luminescence proceeded under conditions which are optimum for oxidative phosphorylation. On the other hand, attention is merited by the fact that bioluminescence in fireflies and bacteria also takes place only in the presence of oxygen and ATP. There is conclusive proof of the bond of bioluminescence with the reaction of oxidation, which may be viewed as an offshoot from the chain of transport of electrons on the level of flavin /5/. If a comparison is made of these data with the results of our investigations, then it can be proposed that bioluminescense is not an exotic phenomenon which is inherent to only individual representatives of the living world, but under specific conditions accompanies any oxidative phosphorylation, even in the tissues of higher animals, where nature, by means of energetic and structural barriers, does not permit the useless waste of valuable chemical energy for radiation. It is possible that the mechanism of participation of free radicals and electronic excited conditions may become more understandable when it is compared with the molecular mechanisms of bioluminescence.

Literature

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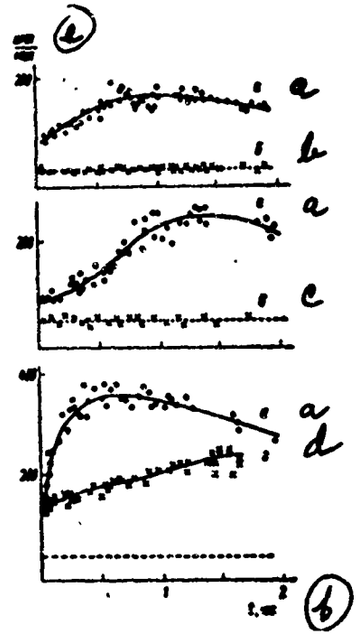

The influence of various factors on the luminescence of a suspension of mitochondria.

a -- complete system (luminescence of mitochondria, suspended in an incubation medium containing 1.2 mg/ml ATP, 2 mg/ml succinic acid, 2.9 mg/ml glutamic acid, 0.005 M $MgCl_2$ tris- and phosphate buffer pH 7.4);

b -- same as a, in the presence of 0.2 mg/ml of EDTA;

c -- same as a, in the absence of ATP;

d -- same as a, in the absence of substrates;

e -- imp/min; f -- t, hours.

On all the graphs the dashes represent the dark background.